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THE SURVIVAL OF OSMOTIC STRESS BY *SYPHAROCHITON PELLISERPENTIS* (MOLLUSCA: POLYPLACOPHORA)

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Osmotic studies on invertebrates may be broadly divided into those on fresh- and brackish-water species, which generally show some degree of osmotic regulation; and those on marine species, which are typically isosmotic with seawater. Many littoral marine animals are however exposed to osmotic changes, and have evolved various mechanical and physiological methods of surviving these stresses. There seem to be no published data on osmotic studies with chitons (a wholly marine group), except a note by Arey and Crozier (1919) on the sensitivity of parts of the body of *Chiton tuberculatus* to osmotic stimulation. Indeed Robertson (1964, p. 284) states, "complete gaps exist for whole classes such as the Amphineura and Scaphopoda."

The subject of this investigation is the common New Zealand chiton *Sypharochiton pelliserpentis* (Quoy and Gaimard, 1835). This is the name at present in general use (Knox, 1963; Morton and Miller, 1967), but there is unpublished work (Johns, 1960) suggesting that the genus *Sypharochiton* (Thiele, 1893) should be relegated to *Chiton* (Linnaeus, 1758). *S. pelliserpentis* is a very common littoral animal on rocky shores throughout New Zealand. It penetrates harbors and estuaries to a certain extent, though this does not bring it into contact with seawater more dilute than about 30‰S. It occupies a broad vertical range on the shore, from open rock to small water-filled hollows, depressions and crevices. These small bodies of water have been found by the present author to be subject to salinity fluctuations of at least 14‰ to 45‰S over very short time periods. The chitons may be exposed to severe osmotic stress for periods of up to 10 hours. The problem, then, is how *S. pelliserpentis* survives these fluctuations in the probable absence of any efficient osmoregulatory ability.

Chitons normally live permanently attached to hard substrates, detachment being the result only of some external force. Unlike some species, *S. pelliserpentis* is unable to right itself when detached and placed upside down on a flat surface. For these reasons the experiments reported here were carried out on animals firmly attached to glass substrates, but some comparative weight change experiments were performed on unattached animals. The salinity conditions used spanned the extreme range recorded in the field, and experiments continued for 24 hours, to exceed the exposure time naturally possible.

Methods

To minimize possible intraspecific adaptive differences, adult animals were always removed from one beach at the same vertical level and from the same

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substrate. They were kept in the laboratory in seawater collected from the site at the same time. The salinity of this was determined, with an inductive salinometer or silver nitrate titration, and fell within the range 34.2‰ to 35.7‰S. Hypotonic media were made up by diluting this 100% seawater with the buffer *M*/400 $NaHCO_3$; "0% seawater" was a solution of *M*/400 $NaHCO_3$ in distilled water. Concentrated seawater (150%) was prepared by evaporation. The animals were used within a few days of collection, the experiments being carried out in 4 liters of experimental solution, contained in "Perspex" tanks and continuously aerated with porous stone diffusers. The tanks were covered with "Perspex" lids to reduce evaporation and consequent salinity change.

Survival of the chitons was measured on the basis of recovery after a period in a favorable environment. At the end of each experiment, animals were transferred at once to a tank containing full strength aerated seawater. After 24 hours in this, the recovery medium, the animals were removed, placed on their dorsal surfaces and scored as follows. Animals which were attached at the end of the recovery period, and in which contractile waves began to pass along the foot shortly after being placed on their dorsal surfaces, and which reattached readily, were designated fully alive and scored 2. Those which were unattached at the end of the recovery period, and immobile when placed on their dorsal surface, but which reacted by feeble curling to light mechanical stimulation of the head, foot or gills, were designated moribund and scored 1. Animals unattached at the end of the recovery peroiod, immobile when on their dorsal surfaces, and unreactive to mechanical stimulation, were designated dead and scored 0.

Using this scheme there was little difficulty in deciding the death of any particular animal. The total score for recovered animals was then expressed as a percentage of the possible total had all animals in the group recovered fully.

Animals were weighed in air in groups attached to glass plates, or individually when unattached. After removal from the experimental medium they were blotted with a soft dry towel and weighed together with the glass plate. Unattached animals were similarly removed and blotted, then weighed individually in a polythene sling from the arm of a torsion balance. The weights in every case were expressed as percentages of the initial weight.

Measurements of the freezing-point depression were made on samples of fluid withdrawn from the pericardial cavity. The total osmotic pressure on the pericardial fluid was assumed to be the same as blood (Potts and Parry, 1964). This has been shown to be so for some lamellibranchs and gastropods (Picken, 1937), though more recent work on the fresh-water snail *Viviparus viviparus* showed slight differences in the O.P. of blood and pericardial fluid (Little, 1965). Indeed this would be expected if pericardial fluid is a filtrate of the blood (Harrison, 1962). No differences in the O.P. of the blood and pericardial fluid of three species of littorinid winkles could be found by Todd (1964). In the chiton, with its sluggish circulation (Martin, Harrison, Huston and Stewart, 1958) and probably very low non-electrolyte content of the blood, the assumed isosmoticity of pericardial fluid and blood seemed justified in view of the gross osmotic changes to be investigated here.

The pericardium lies directly beneath shell valves VII and VIII, and fluid was removed directly by piercing the dorsal body wall between these two valves and

inserting a fine "Pyrex" glass capillary tube. The sampling tube was first filled with liquid paraffin, a short column of sample drawn in, followed by more liquid paraffin. In this way duplicate or triplicate sub-samples (0.01 μl to 0.1 μl in volume) were removed from the animal and drawn up the tube between liquid paraffin columns. Difficulty in removing fluid samples was only experienced with animals after prolonged exposure to 150% seawater. Samples visibly contaminated with cell debris or genital products after accidental rupture of the gonad were rejected immediately. The tubes were placed at once on solid carbon dioxide, and could be stored at $-18°$ C without measurable change in melting-point of the sample. Animals suffered irreparable damage and could only be sampled on one occasion.

The freezing-point depressions ($\Delta°$ C) of these samples was determined by allowing them to warm up slowly in a continuously stirred alcohol bath. The frozen samples were viewed with a binocular microscope through crossed "Polaroids". The temperature at which each sample melted was read on a chemical Beckmann thermometer, graduated in 0.01° C divisions, and compared directly with that for distilled water sampled in a same way and included in the bath for each experimental run. The $\Delta°$ C of the seawater media of the animals was similarly determined for each experiment.

Temperature of experiments was controlled by suspending the "Perspex" tanks in a large constant temperature bath. A low temperature of 10° C was used, approximately the lower limit of sea surface temperatures in Auckland (Skerman, 1958). At the upper end of the scale, a temperature of 30° C was used, this temperature being frequently attained or exceeded in small littoral pools during summer. Room temperature during the experiments was 20° C $\pm$ 2° C.

Results

Survival and weight changes in various salinities

The percentage survival of attached *Sypharochiton pelliserpentis* (groups of 10), in 0%, 50%, 100% and 150% seawaters, after 2, 6, 10 and 24 hours exposure was determined. The experiments were repeated at three temperatures, 10° C $\pm$ 0.1° C, 20° C $\pm$ 0.5° C and 30° C $\pm$ 0.1° C. The data for survival at a controlled temperature of 20° C are taken to indicate survival at room temperature. Survival was 100% after 24 hours in concentrated (150%) or normal (100%) seawaters at 20° C and 30° C, but between 95% and 100% at 10° C. In 50% seawater, survival was 80 to 85% after 10 and 24 hours exposure at 10° C, but was 100% at 20° C. At 30° C, survival after 10 hours was 85%, but after 24 hours was only 5%. Freshwater (0% seawater) resulted in 85% survival after 10 hours and 15% survival after 24 hours at 10° C. At 20° C in 0% seawater, mortality occurred between 10 and 24 hours exposure, there being only 10% survival after this time. In 0% seawater at 30° C, survival was down to 80% after only 2 hours, and there were no survivors after 6 hours in this medium at this temperature.

Summarizing these results, under laboratory conditions *S. pelliserpentis* survived well in 150% and 100% seawaters for 24 hours at temperatures of 10° C to 30° C. Half strength (50%) seawater caused significant mortality (greater than 50%) only at 30° C and after exposures longer than 10 hours. Freshwater (0%

seawater) caused significant mortality only after 10 hours at 10° C and 20° C, but after 2 hours at 30° C.

Mean weight changes of groups of attached *S. pelliserpentis* in several experimental seawaters are shown in Figure 1 (each point is the mean of 5 animals). A rapid increase in weight resulted from transfer to hypotonic media. In 75%

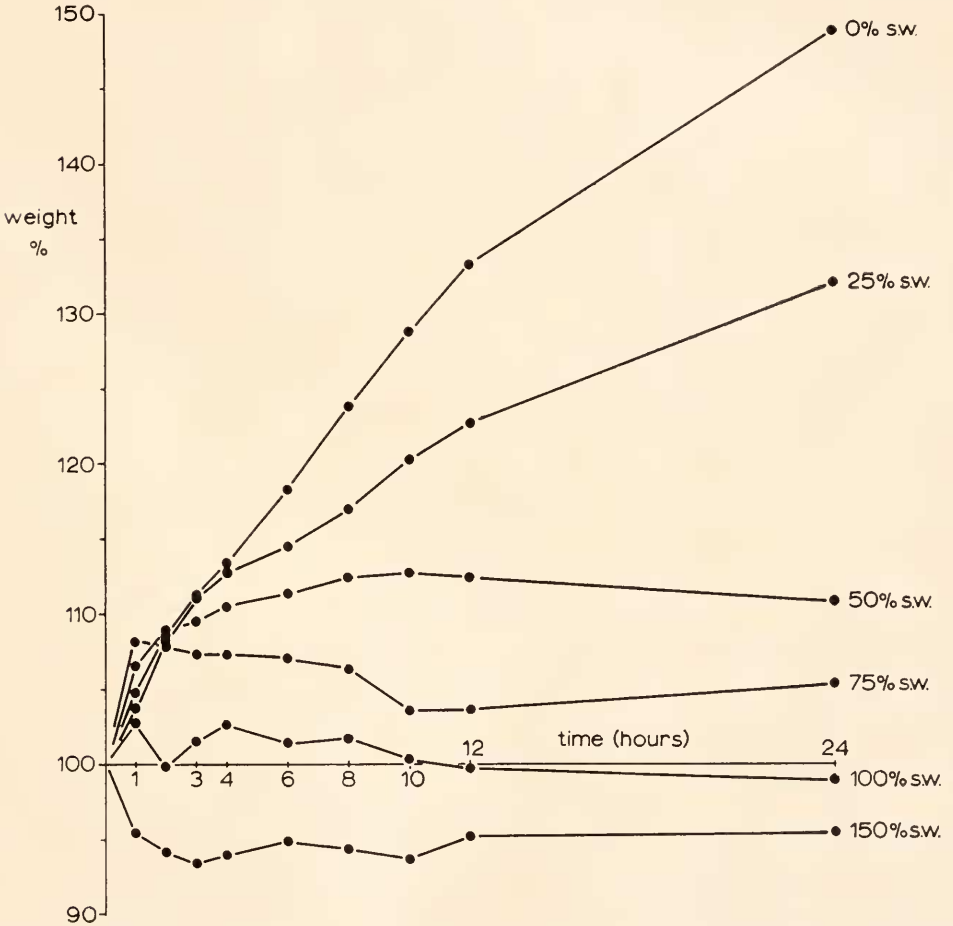

FIGURE 1. Mean weight changes (as % of initial weight) of groups of attached *S. pelliserpentis* in several experimental seawaters. Room temperature.

seawater weight equilibrium was reached and followed by a decrease towards the initial weight. Animals in 50% seawater reached equilibrium more slowly and showed a small decrease towards the initial weight. When transferred to more dilute media (25% and 0% seawaters) animals did not attain weight equilibrium within 24 hours.

Control animals, remaining in the stock 100% seawater but weighed at the same intervals as the experimental animals, showed small weight fluctuations about

the initial weight. In hypertonic seawater (150%) a small weight loss was recorded, not proportional to the weight increase in 50% seawater.

These experiments were repeated with unattached animals and the results are shown in Figure 2. Here the initial weight increase in hypotonic media was more rapid. Survival of these animals after recovery was identical with that of the attached animals, except that after exposure to 25% seawater, survival was only 80%. No significance is attached to this difference.

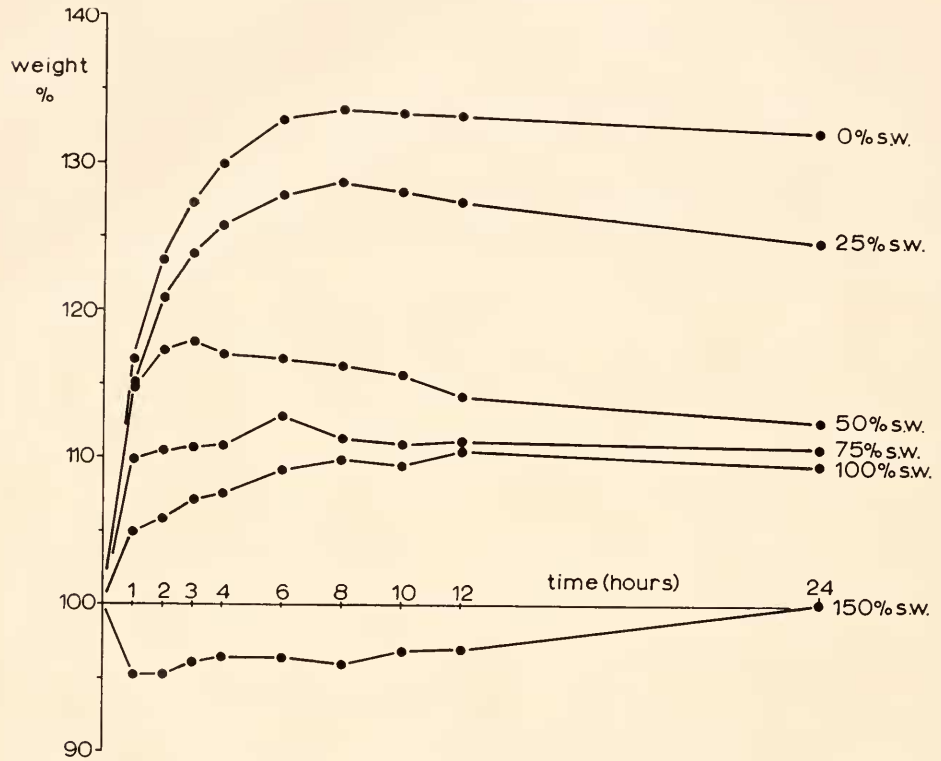

FIGURE 2. Mean weight changes (as % of initial weight) of unattached *S. pelliserpentis* in several experimental seawaters. Room temperature.

Unattached control animals consistently showed a steady weight increase when no osmotic gradient was present. An interpretation of this is based on the animals' behavior. The initial weight was derived from animals just detached from the tank wall and blotted while extended. Following this the animals tended to curl while in air. On return to the experimental tank some extended and actively flexed backwards, others remained partly curled. As the experiment progressed and after further disturbances for weighing, all animals showed an increasing tendency to remain curled. The gradual weight increase shown in Figure 2 could be due to this progressive curling tendency, trapping water in the mantle groove. Attached chitons in the same control situation often showed slight fluctuations, but never as great or as consistent as those of unattached animals.

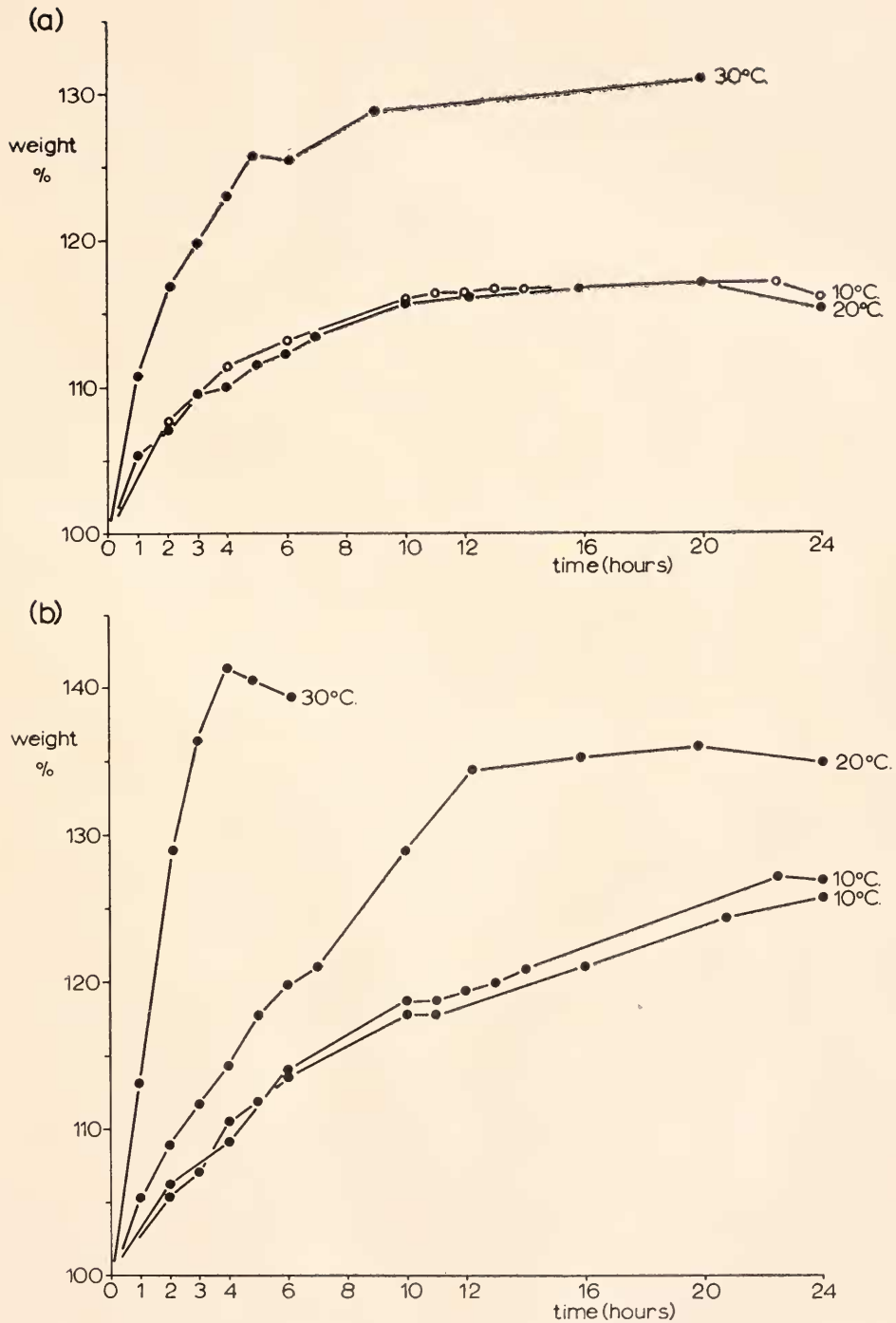

FIGURE 3. Effect of temperature on weight changes of groups of attached *S. pelliserpentis* in (a) 50% seawater, and (b) 0% seawater.

Hypertonic seawater (150%) caused a small weight loss, not proportional to the weight gain in 50% seawater. After 24 hours in this medium, animals almost regained their initial weight.

The results of repeating these weight change experiments on attached animals in hypotonic media at three controlled temperatures, are plotted in Figure 3. At 20° C (Fig. 3b), apparent weight equilibrium in 0% seawater was reached, contrasting with the absence of weight equilibrium in similar conditions in the experiment shown in Figure 1. At this stage, considerable mortality would have resulted from the experiment.

Osmotic adjustment in various salinities

Experiments on the relation of the internal and external osmotic pressures in hypotonic seawaters are summarized in Figure 4. Each point is the mean value for Δ° C of 8 to 10 animals, and the time after transfer to hypotonic conditions is shown on each curve.

Clearly the osmotic gradient between the animal and its surrounding medium decreases rapidly with time. Adjustment takes place. Increase in temperature accelerates the rate of adjustment.

It is shown in Figure 5 that pericardial fluid is normally isosmotic with seawater between 10° C and 30° C. In 150% seawater the internal concentration adjusts very rapidly, usually within two hours of transfer.

Behavioral reaction to salinity change

Unattached animals remained curled after repeated weighings; this took place regardless of salinity and was assumed to be a result of disturbance. It has been discussed relative to weight changes of control groups of animals.

With attached animals, two aspects of behavior were considered; the initial movement of the animals after transfer to the medium, and the length of time for which movement continued. Normally, when animals attached to glass plates were immersed in 100% seawater, they began at once to move about and crawl over one another. Continuous movement soon stopped but intermittent activity occurred over 24 hours. If repeated weighings of them were made, the same pattern of movement was repeated but the duration of continuous movement decreased after each return to the water.

In 75% seawater movement was almost normal, the animals showing some activity for most of a 24-hour period. When transferred direct to 50% seawater however, animals showed more vigorous initial movements, raising and lowering the girdle, turning to and fro, and raising the head. They remained active in this medium for an hour or two, finally attaching firmly in one place. Most were still attached in the same place after 24 hours.

In 0% seawater the initial movements were greatly exaggerated, animals immediately moving, raising the girdle, and flexing backwards exposing the anterior ventral surface. This continued for a few minutes only, after which they attached very firmly, not to move again until the termination of the experiment. After 24 hours in this medium the animals were frequently detached—forced off the substrate by the swelling of the foot.

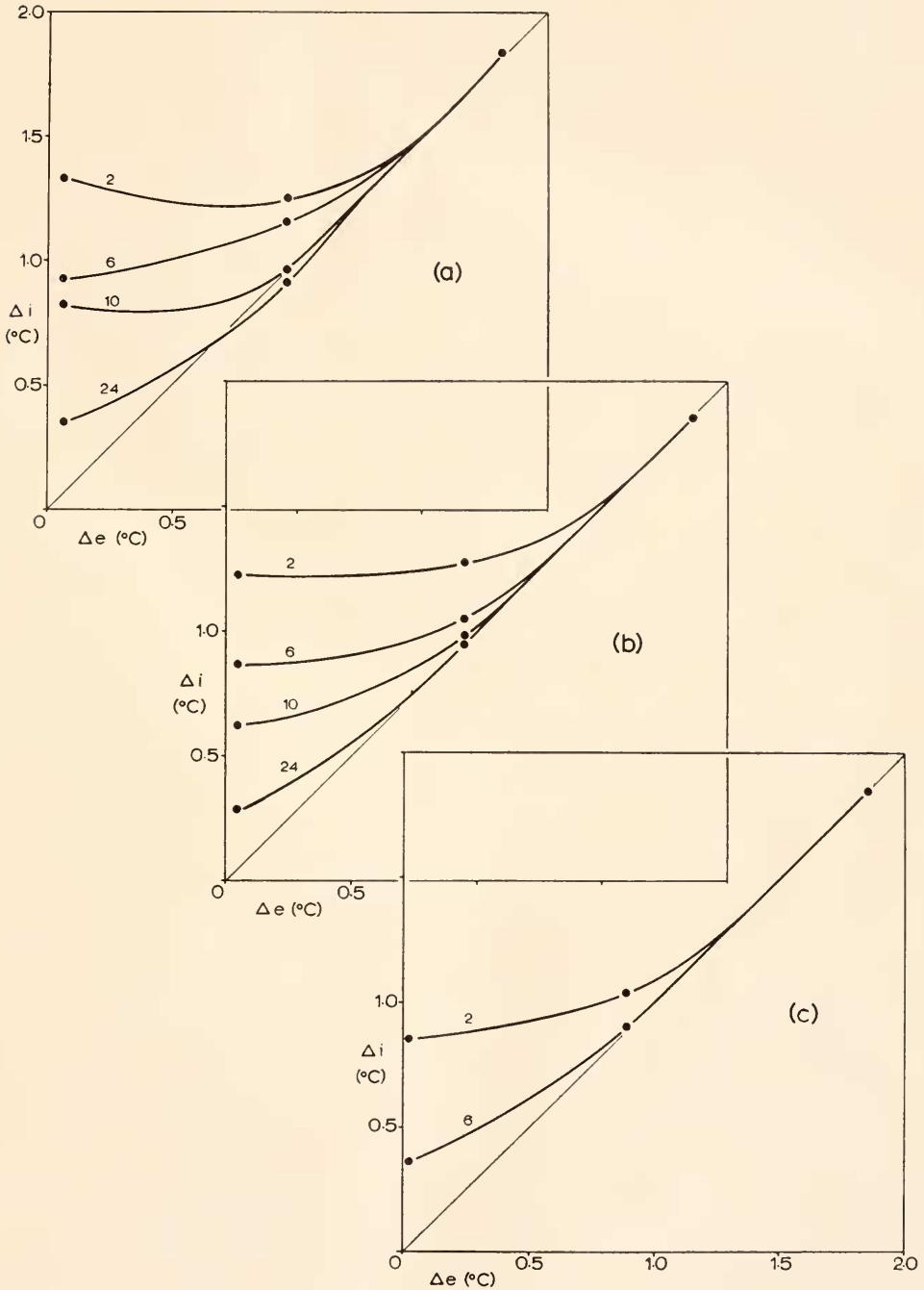

FIGURE 4. Adjustment of pericardial fluid osmotic pressure to that of the external medium at three different temperatures. Time (hours) after transfer to the external medium marked on each curve. (a) 10° C ± 0.1° C; (b) 20° C ± 0.5° C; (c) 30° C ± 0.1° C.

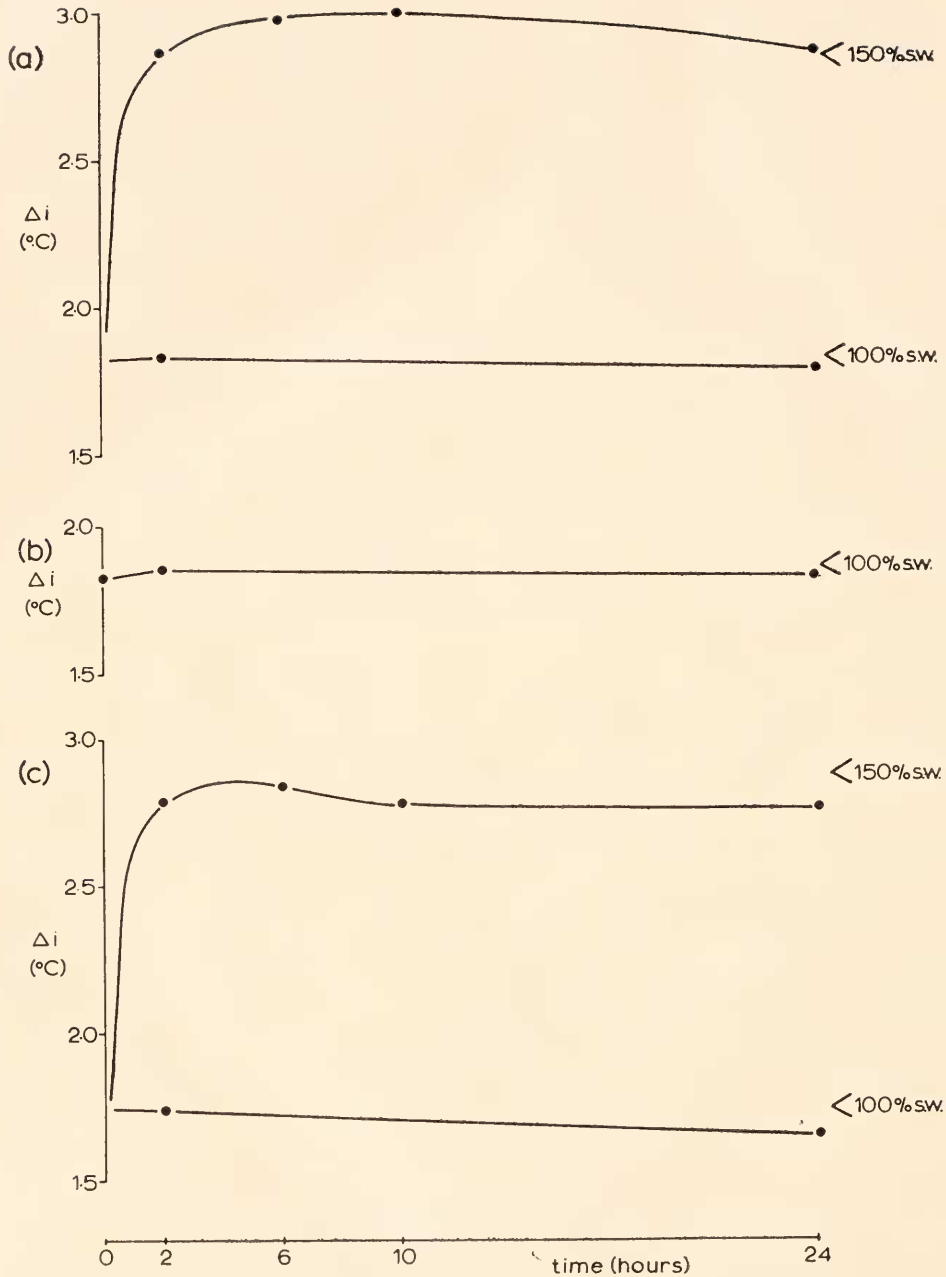

FIGURE 5. Pericardial fluid osmotic pressure in 100% seawater for 24 hours (Controls), and after transfer to hypertonic medium (150% seawater) at two temperatures. (a) 10° C ± 0.1° C; (b) 20° C ± 0.5° C; (c) 30° C ± 0.1° C.

In 150% seawater hardly any movement occurred.

Few faeces were found in tanks of 50% and 0% seawater, while control animals defecated freely. Animals transferred from these conditions to 100% seawater for recovery usually defecated within a few minutes.

Discussion

As all control groups of animals have shown, *Sypharochiton pelliserpentis* is normally in osmotic equilibrium with the surrounding seawater. Differences between the Δi and Δe after 24 hours in normal seawater shown in Figure 5 were found not to be significant ($P = 0.5$).

Unattached animals (Fig. 2) showed a rapid weight increase when transferred to hypotonic media, followed by weight equilibrium and some weight regulation. The effect of firm attachment in reducing the surface area available for diffusion can account both for the lower rate of weight increase, and the greater weight increase eventually reached by attached animals. Firstly the rate of water entry and consequent weight increase is reduced, and secondly salt loss is presumably also reduced so that correspondingly more water enters before weight equilibrium can be attained.

Both attached and unattached animals in dilutions down to 50% seawater seem capable of limited, active weight regulation. In seawater/sucrose mixtures isosmotic with seawater, *S. pelliserpentis* lost weight. This implies salt loss from the animal and probably means that salt loss in dilute media contributes to a passive weight regulation.

There is no evidence that *S. pelliserpentis* is in any way able to regulate its internal osmotic concentration, except by reducing contact with the external medium and thus delaying equilibrium. In 150% and 50% seawaters, osmotic equilibrium was reached rapidly. In 0% seawater, dilution of the internal fluid was very rapid, but mortality occurred before equilibrium was reached. No definite behavioral reaction to a hypertonic medium was recorded. As no mortality resulted from exposure to this medium for up to 24 hours, hypertonicity at this level (150% seawater) was assumed to be of little physiological importance to the animal. Chitons in 150% seawater were easily detached from the substrate, a result presumably of the loss of body fluid. It is probable that loss of body fluid could itself cause loss of locomotory ability.

S. pelliserpentis is able to detect and react to reduced salinities, as can *Chiton tuberculatus* (Arey and Crozier, 1919) and many other molluscs. That clamping down hard to the substrate is more rapid and complete the more dilute the medium, could well account for the situation shown in Figure 1. Here 1 hour after transfer, weight increase was greater in 75% than in 50%, which in turn was greater than in 25% or 0% seawaters.

Weight loss in 150% seawater is smaller than would be expected. Possibly only a limited weight loss can occur, a limit which is quickly reached. This would be possible if blood volume is small (44% in *Cryptochiton stelleri;* Martin *et al.,* 1958), and if water and/or salts are unable to leave the body tissues, which would occur if the tissue cells have a limited permeability, or can otherwise restrict the outflow of water and ions.

In effect, the osmotic response of *S. pelliserpentis* in the absence of any osmoregulatory ability may be considered as a compromise. This chiton is physically well adapted to reduce contact with the environment to a minimum during adverse conditions. Reduction in the rate of osmotic adjustment, coupled with a considerable degree of tolerance of dilution of body fluid is sufficient to ensure survival of the animals for periods of over 10 hours—the maximum exposure time possible. Only very low salinities and high temperature (the least likely combination) could cause mortality in a shorter time. The range of salinity fluctuations recorded in the field (14‰ to 45‰S) suggests that exposure to osmotic extremes is not likely to limit this species in its exploitation of littoral habitats. A limited, active volume regulation is effective in hypotonic seawaters down to about 50% seawater.

This type of osmotic response—with water inflow, some volume regulation, osmotic equilibrium—is typical of many worms, for instance the sipunculid *Dendrostomum zostericolum* (Gross, 1954). Other worms show in addition, osmoregulation; *Onuphis magna* (Ebbs and Staiger, 1965), *Nereis diversicolor* (Jørgensen and Dales, 1955–1957). Some soft-bodied molluscs such as *Aplysia* (Bethe, 1930), show volume regulation, and possibly weak osmoregulation (van Weel, 1957), while others such as *Onchidium* (Dakin and Edmonds, 1931) do not. The habit of mechanically restricting contact with adverse conditions is found commonly in bivalves such as *Mytilus edulis* (Maloeuf, 1938) and *Scobicularia plana* (Freeman and Rigler, 1957), and also the littorinid gastropods *Littorina littorea, L. littoralis* and *L. saxatilis* (Todd, 1964).

The limpet *Acmaea limatula* after weight increases in 50% seawater showed no tendency to return to the initial weight (Segal and Dehnel, 1962). Its increase in body water in 50% seawater was approximately equal to the decrease in 150% seawater. This has been shown here not to be the case in *S. pelliserpentis*. *Acmaea* did not osmoregulate in salinities from 25% to 150% seawater. The pulmonate limpet *Siphonaria pectinata* is also unable to osmoregulate over a wide salinity range (McAlister and Fisher, 1968). In *Siphonaria* it was shown that undisturbed attachment to the substrate was very important in resisting osmotic stress.

Gilbert (1959) showed that the Δ° C and the composition of the blood of the shore crab *Carcinus maenas* are correlated with the size of the animal. The results summarized in Figure 4 were analyzed for the effect of size on the O.P. of pericardial fluid. No correlation was shown after equilibrium had been reached, nor when the osmotic gradient was steep and the animals still adjusting. These results agree with those for littorinids (Todd, 1964) where it was shown that size had no effect on osmotic balance.

Work with the American West Coast chiton genus *Mopalia* (Barnes, 1965, unpublished student report) has indicated that animals of this genus are osmoconformers in 125% to 50% seawater media.

Sypharochiton pelliserpentis then is a marine animal which on the shore colonizes habitats bringing it into contact with wide salinity fluctuations for short periods of time. In the absence of any extracellular osmoregulatory mechanism, the animal is able to detect salinity changes and react by clamping down to the substrate, isolating its internal fluids from the external medium and reducing the rate of osmotic adjustment. Though capable of a limited active weight regulation, the

animal relies for survival primarily on its ability to tolerate, physiologically, considerable swelling and dilution of its internal medium.

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Summary

1. The survival, weight changes and pericardial fluid osmotic pressure changes of the chiton *Sypharochiton pelliserpentis*, when subjected to hypo- and hypertonic media (0% to 150% seawater), have been measured. Three experimental temperatures were used, 10, 20 and 30° C, and the size of the animals was taken into account.

2. The chiton was found to be an osmoconformer, its pericardial fluid O.P. adjusting to that of the medium within the range 50% to 150% seawater. It was able to survive these changes for periods of at least 24 hours. In freshwater (0% seawater) dilution of the internal fluid was more rapid, but mortality occurred before equilibrium was reached.

3. Attachment to the substrate is significant in restricting the rate of water entry. The animal detects hypotonic salines and reacts accordingly by clamping down tightly. A limited weight regulation was observed in dilutions down to 50% seawater.

4. Temperature affects the osmotic response, higher temperatures increasing the rate of osmotic adjustment, and the mortality rate.

5. Field measurements suggest that microhabitat osmotic fluctuations are unlikely to limit the distribution of *S. pelliserpentis* on the shore.

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